US Serial No: 10/812,831

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/68114 A1

(51) International Patent Classification[7]: A61K 38/00, 38/06, C07K 5/00, 5/10, A23C 9/12

(21) International Application Number: PCT/US01/07530

(22) International Filing Date: 9 March 2001 (09.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/188,497 10 March 2000 (10.03.2000) US

(71) Applicant *(for all designated States except US)*: **MONSANTO COMPANY** [US/US]; 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TJOENG, Foe, Siong** [US/US]; 753 Castle Ridge Drive, Ballwin, MO 63021 (US). **GUAN, Zhonghong** [CN/US]; 2040 Parasol Drive, Chesterfield, MO 63017 (US). **LI, Wei** [US/US]; 12057 Nancy Lee Drive, St. Louis, MO 63146 (US). **MANDRELL, Kathy** [US/US]; 401 George Street, Alton, IL 62002 (US). **LIU, Min** [CN/US]; 2552 Hidden Meadow Lane, St. Louis, MO 63021 (US). **CHEN, Xiangra** [CN/US]; 1708 Ekkon Ridge Court, Chesterfield, MO 63017 (US).

(74) Agents: **BLOSSER, G., Harley**; Senniger, Powers, Leavitt & Roedel, One Metropolitan Square, 16th Floor, St. Louis, MO 63102 et al. (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/68114 A1

(54) Title: NOVEL PEPTIDES WITH ANTI-HYPERTENSIVE ACTIVITY

(57) Abstract: Provided are novel tri-peptides which exhibit anti-hypertensive activity. These anti-hypertensive peptides may be used as active ingredients in pharmaceutical compositions, dietary supplements and food ingredients. The present invention also relates to using such novel anti-hypertensive peptides for methods of treatment and prophylaxis of primary hypertension as well as hypertension associated with myocardial infarction, left ventricular systolic dysfunction, diabetes mellitus, progressive renal failure and congestive heart failure.

NOVEL PEPTIDES WITH ANTI-HYPERTENSIVE ACTIVITY

Cross References of Related Applications

This application claims priority from U.S. Provisional Application No. 60/188,497, filed March 10, 2000, which is hereby incorporated by reference in its entirety for all purposes.

Field of Invention

This invention relates to novel tripeptides which exhibit anti-hypertensive properties. These novel anti-hypertensive tripeptides may be used as active ingredients in food products, pharmaceutical compositions, and dietary supplements. The present invention also relates to methods of treatment and prophylaxis of hypertension, myocardial infarction, left ventricular systolic dysfunction, diabetes mellitus, progressive renal failure and congestive heart failure using such novel tripeptides.

Background of the Invention

Approximately 60 million people in the United States and 170 million people worldwide suffer from hypertension, which is an abnormally increased blood pressure. Hypertension is generally clinically defined as a systolic blood pressure greater than 140 mmHg or a diastolic blood pressure greater than 90 mmHg. Hypertension is the primary risk factor for coronary, cerebral, and renal vascular diseases which cause over half of all deaths in the United States. The widespread awareness of the danger of elevated blood pressure has become the most frequent reason for visits to physicians.

No single or specific cause is known for the hypertension referred to as primary (essential) hypertension. Primary hypertension has been attributed to such causes as hemodynamic pattern, genetic predisposition, vascular hypertrophy, hyperinsulinemia, defects in cell transport of binding, defects in the reninangiotensin system

(low-renin or high renin hypertension) and along with insulin, angiotensin and natriuretic hormone, catecholamines arising in response to stress are known to be pressor-growth promoters. Increased sympathetic nervous activity may raise the blood pressure in a number of ways, for example, either alone or in concert with stimulation of renin release by catecholamines, causing arteriolar and venous constriction, by increasing cardiac output, or by altering the normal renal pressure-volume relationship. Also, recent evidence demonstrates that sodium has a causal role in the genesis of hypertension. Primary hypertension is also associated with, for example, obesity, sleep apnea, physical inactivity, alcohol intake, smoking, diabetes mellitus, polycythemia and gout. Secondary forms of hypertension may arise from oral contraceptive use and parenchymal renal disease: renovascular hypertension caused by, for example, atherosclerotic disease, tumors (renin-secretory tumors); Cushing's Syndrome; heart surgery; and pregnancy. Chronic hypertension and renal disease during pregnancy may progress into eclampsia, a primary cause of fetal death. Essential hypertension is a relatively common disease state in humans. This disease has been associated with the early onset of coronary disease, kidney failure and stroke. Essential hypertension is generally asymptomatic and has been termed a silent killer.

Among a number of factors for regulating blood pressure, the renin-angiotensin system plays an important role in salt-water homeostasis and the maintenance of vascular tone; stimulation or inhibition of this system respectively raises or lowers blood pressure, and may be involved in the etiology of hypertension. Hall, J.E., and Guyton, A.C. (1990), *In Hypertension: Pathophysiology Diagnosis and Management*, (Raven Press, Ltd., New York), pp.1105-1129. Angiotensin converting enzyme (ACE) plays an important role in the renin-angiotensin system. ACE acts on angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), which is formed by decomposition of angiotensin secreted by

the liver, by an enzyme, renin, produced in the kidney, and converts it to angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). Angiotensin II increases blood pressure by contracting the smooth muscles of the blood vessel walls and promoting secretion of aldosterone by action on the adrenal cortex. Thus, ACE acts to create the blood pressure increasing enzyme of angiotensin II. Additionally, ACE decomposes and inactivates a protein called bradykinin, dilates the blood vessels and lowers blood pressure. Therefore, a common method of decreasing blood pressure in an individual is to inhibit the activity of ACE.

Several pharmaceuticals have been studied for their ability to reduce blood pressure. Much focus has been directed on developing compounds which inhibit the function of ACE in the renin-angiotensisn mechanism. However, while the inhibition of ACE affects blood pressure, reduction in blood pressure often can not be explained by the inhibition of ACE alone. Thus antihypertensive compounds may reduce blood pressure through other mechanisms or through a combination of physiologic mechanisms.

Current pharmaceutical treatments for essential hypertension include diuretics, beta-blockers, angiotensin converting enzyme inhibitors and calcium antagonists. Many ACE inhibiting substances are known and commonly used for the purpose of decreasing blood pressure in patients. Among commonly used ACE inhibiting pharmaceuticals is the synthetic chemical product known as captopril (D-2-methyl-3-mercaptopropanoyl-L-proline), which is an oral hypotensive agent. Currently available anti-hypertensive agents are not without side effects such as the elevation of blood lipids and glucose. The elevation of blood lipids and glucose by these agents has been suggested as a reason why anti-hypertensive agents have not demonstrated any benefit to patients being monitored in death rate studies. Also, special care must be taken to monitor the safety of synthetic chemical products for anti-hypertensive use.

Dietary supplements derived from natural sources or synthesized, as well as pharmaceutical compositions, are important to control the blood pressure of patients suffering from hypertension. Recently, a number of functional peptides derived from milk, soy, corn, gelatin, wheat, and fish protein have been identified as having functions relating to physiological regulation and ACE inhibition. *See e.g.*, U.S. Patent Nos. 5,238,932 and 5,071,955. ACE inhibiting substances isolated from various foods and microorganisms are being investigated for their potential as anti-hypertensive agents. (Kunio Suetsuna, "Hakko to Kogyo" (Fermentation and Industry) 46 (No. 3), 179-182 (1988)). Furthermore, ACE inhibiting substances may be derived from casein and corn seed proteins (*See* Susumu Maruyama, Biosciences and Industry 47 (No. 11), 38-42 (1989); Susumu Maruyama et al., Lecture Gists for the 1988 Year Great Annual Meeting of Nippon Hakko Kogaku Kai (Japan Fermentation Engineering Society), p.23 (1988); Susumu Maruyama et al., Lecture Gists for the 1989 Year Meeting of Nippon Nogei Kagaku Kai (Japan Society for Bioscience, Biotechnology and Agrochemistry), p.8 (1989); Shinsuke Miyoshi et al., Gists for the 1989 Year Meeting of Nippon Eiyo Shokuryo Gakki (Japan Nutritional and Food Society) p.113 (1989); and Shinsuke Miyoshi et al., Nippon Nogei Kagaku Kaishi (Journal of Japan Agricultural Chemistry Society), 64(3), 555, 1990 (Lecture Gists for the 1990 Year Great Annual Meeting)) and fish meat protein from sardines, tuna and bonito (*See* Hiroyuki Ukeda, Nippon Nogei Kagaku Kaishi (Journal of Japan Society for Bioscience, Biotechnology, and Agrochemistry), 66(1), 25-29 (1992); Astawan et al., "Effects of Angiotensin I-Converting Enzyme Inhibitory Substances Derived from Indonesian Dried-salted fish on Blood Pressure of Rats," Biosci.Biotech.Biochem., 59 (3), 425- 429, 425 (1995)). Some of these natural ACE inhibitory peptides derived from food products have been reported as effective in reducing hypertension.

Recently, two tripeptides reported as having strong ACE inhibiting activity, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) have been derived from lactic acid bacteria-fermented milk. *See* Nakamura et al., J. Dairy Sci. 78:777-783 (1995). Furthermore, these tripeptides have been reported to exhibit strong antihypertensive effect in spontaneously hypertensive rats (SHR). See Nakamura, et al., J. Dairy Sci., 78:1253-1257 (1995). However, since the tripeptides are produced by proteinase which is produced by lactic acid bacteria as lactic acid fermentation proceeds in milk, the resulting amount of tripeptides tends to vary depending on the conditions of fermentation. It is thus difficult to obtain a consistent amount of the tripeptides.

These peptides isolated from milk, soy, corn, gelatin, wheat and fish that have been reported as having ACE inhibitory properties are proposed for practical use as anti-hypertensive agents having low toxicity and great safety. However, most of these anti-hypertensive substances are contained only in small amounts in such natural products and therefore sufficient effect cannot be expected in practical oral intake.

Additionally, many peptides do not have strong anti-hypertensive effects even if the peptides have strong ACE inhibition activity. Also, some peptides exhibit anti-hypertensive activity without exhibiting ACE inhibitory activity. Therefore, ACE inhibition is but one potential pharmacological mechanism of many of these peptides, or alternatively, the peptides have to be metabolized to other active forms which reduce blood pressure by ACE inhibition.

Therefore, there is significant interest in obtaining anti-hypertensive peptides which can be produced in an industry stable manner and amount and which are effective in oral dosage and have low toxicity and great safety without appreciable side effects associated with existing anti-hypertensive pharmaceutical products. Currently, there are few non-pharmaceutical products for treating hypertension in the nutritional or dietary supplement markets. There is a

need for an effective method of treating hypertension which utilizes anti-hypertensive peptides that may be conveniently administered, preferably as a dietary supplement, do not have appreciable side effects, are easy to manufacture in a stable manner and amount, and are available at a low cost.

Summary of the Invention

Accordingly, it is an object of the present invention to provide novel anti-hypertensive peptides to be used for pharmaceutical compositions, dietary supplements, food ingredients, and foods for specified health uses of reducing and inhibiting hypertension and disease states associated with hypertension at a low cost with no appreciable side effects.

A further object of the present invention is to provide an anti-hypertensive composition comprising an anti-hypertensive peptide or its acceptable acid addition salt or base salt thereof, together with a pharmaceutically suitable diluent.

The present invention provides an anti-hypertensive peptide selected from the group of peptides consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, and His-Pro-Pro.

Also, the present invention provides an anti-hypertensive agent comprising an effective amount of a peptide selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, and His-Pro-Pro or a pharmacologically acceptable acid addition salt thereof.

Additionally, the present invention provides a food composition suitable for treatment or prophylaxis of hypertension comprising a nutritional substance and a peptide selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, and His-Pro-Pro or a pharmacologically acceptable acid addition salt thereof in an anti-hypertensively effective amount.

Yet a further object of the present invention is to provide methods of treating or prophylaxis of essential hypertension as well as hypertension associated with myocardial infarction, left ventricular systolic dysfunction, renal impairment or failure, congestive heart failure, and diabetes mellitus which comprises administering an effective amount of an anti-hypertensive peptide in a food or pharmaceutical composition.

Other features of the present invention will be in part apparent to those skilled in the art and in part pointed out in the detailed description provided below.

Abbreviations and Definitions

To facilitate understanding of the invention, a number of terms as used herein are defined below:

The amino acid residues are abbreviated herein according to their single letters: A represents alanine; R represents arginine; N represents asparagine; D represents aspartic acid; C represents cysteine; Q represents glutamine; E represents glutamic acid; G represents glycine; H represents histidine; I represents isoleucine; L represents leucine; K represents lysine; M represents methionine; F represents phenylalanine; P represents proline; S represents serine; T represents threonine; W represents tryptophan; Y represents tyrosine; and V represents valine.

As used herein, "ACE" shall mean angiotensin converting enzyme.

As used herein, the terms "treatment" or "treating" relate to any treatment of hypertensive disease or diseases related to hypertensive and include: (1) preventing hypertension from occurring in a subject who may be predisposed to the disease but who has not yet been diagnosed as having it; (2) inhibiting the disease, i.e., arresting its development; or (3) ameliorating or relieving the symptoms of the disease, i.e., causing regression of the hypertensive state. Diseases related to hypertension, which

may be treated by the methods, compounds and compositions of this invention, include, but are not limited to, myocardial infarction, left ventricular systolic dysfunction, renal impairment or failure, congestive heart failure and diabetes mellitus.

As used herein, the terms "anti-hypertensively effective amount" shall mean an amount sufficient for treatment and prophylaxis of hypertension; an anti-hypertensively effective amount of the peptide of this invention would be an amount necessary to reduce, inhibit, or prevent hypertension in a subject.

As used herein, the terms "substantially pure" or "isolated", when referring to proteins and polypeptides, denotes those polypeptides that are separated from proteins or other contaminants with which they are naturally associated. A protein or polypeptide is considered substantially pure when that protein makes up greater than about 50% of the total protein content of the composition containing that protein, and typically, greater than about 60% of the total protein content. More typically, a substantially pure protein will make up from about 75 to about 90% of the total protein. Preferably, the protein will make up greater than about 90%, and more preferably, greater than about 95% of the total protein in the composition.

As used herein, the terms "ingestible composition" are defined as foodstuffs and pharmaceutical products and preparations. Typical ingestible compositions, which include foodstuffs and pharmaceutical preparations of the present invention, are, for example, beverages, (including soft drinks, carbonated beverages, ready to mix beverages and the like), infused foods (e.g. fruits and vegetables), sauces, condiments, salad dressings, fruit juices, syrups, desserts (including puddings, gelatin, icings and fillings, baked goods and frozen desserts, such as ice creams and sherbets), chocolates, candies, soft frozen products (such as soft frozen creams, soft frozen ice creams and yogurts, soft frozen toppings, such as dairy or non-dairy whipped

toppings), oils and emulsified products (such as shortening, margarine, mayonnaise, butter, cooking oil, and salad dressings), intermediate moisture foods, (e.g. rice and dog foods) and the like.

Description of the Figures

Figure 1 is a graph representing the effects of oral doses of Asp-Lys-Pro and Tyr-Lys-Pro on Systolic Blood Pressure (Tail Cuff Method) administered to spontaneous hypertensive rats as discussed in Example 8 for *in vivo* dose response studies.

Figure 2 is a graph representing the effects of oral doses of His-Pro-Pro on Systolic Blood Pressure (Tail Cuff Method) administered to spontaneous hypertensive rats as discussed in Example 8 for *in vivo* dose response studies.

Figure 3 is a graph representing the effects of oral doses of Glu-Lys-Pro on Systolic Blood Pressure (Tail Cuff Method) administered to spontaneous hypertensive rats as discussed in Example 8 for *in vivo* dose response studies.

Figure 4 is a graph representing the effects of oral doses of Glu-Ala-Pro and Gly-Lys-Pro on Systolic Blood Pressure (Tail Cuff Method) administered to spontaneous hypertensive rats as discussed in Example 8 for *in vivo* dose response studies.

Figure 5 is a graph representing the effects of oral doses of Asp-Ala-Pro on Systolic Blood Pressure (Tail Cuff Method) administered to spontaneous hypertensive rats as discussed in Example 8 for *in vivo* dose response studies.

Detailed Description of the Invention

Applicants have discovered novel tripeptides which exhibit anti-hypertensive activity and effectively reduce blood pressure without having substantial adverse side effects or significantly altering normal blood pressure.

These anti-hypertensive peptides include Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, and His-Pro-Pro. Because these peptides have significant anti-

hypertensive activity, they are also useful in the treatment and prophylaxis of hypertension, left ventricular systolic dysfunction, myocardial infarction, diabetes mellitus and progressive renal impairment or failure as well as other diseases caused by or associated with hypertension. The peptides of the present invention may be either chemically synthesized or derived from food protein digest products. The method of action of these peptides remains unknown. Applicants hypothesize that the mechanism of action may be ACE inhibition, or alternatively the peptides may have to be metabolized to other active forms which reduce blood pressure by ACE inhibition. Other pharmaceutical methods of action may cause the anti-hypertensive activity of these peptides.

The present invention also provides pharmaceutical compositions comprising such anti-hypertensive peptides Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, and His-Pro-Pro or pharmacologically acceptable acid addition salts or base salts thereof. The salt may be a pharmaceutically acceptable salt, including an inorganic acid salt, such as hydrochlorate, sulfate or phosphate, or an organic acid salt, such as citrate, maleate, fumarate, tartarate or lactate. Additionally, acid addition salts include pharmacologically acceptable acid (inorganic acid or organic acid) addition salts, such as hydrochloride, hydrobromide, sulfate, nitrate, acetate, benzoate, maleate, fumarate, succinate, tartrate, citrate, oxalate, methanesulfonate, tolunesulfonate, aspartate, glutamate, etc. Furthermore, alkali and alkaline earth metal and ammonium salts include pharmacologically acceptable salts, such as sodium, potassium, calcium and ammonium salts, and organic base salts include pharmacologically acceptable salts, such as basic amino acid salts including lysine and ornithine salts.

The anti-hypertensive peptides of the present invention may be synthesized using either solid-phase peptide synthesis, by classical solution peptide synthesis, as well

as by liquid-phase peptide synthesis using a soluable oligomeric support. Using Val-Pro-Pro, Enalapril and Lisinopril as starting templates, several series of peptide analogs such as X-Pro-Pro, X-Ala-Pro, and X-Lys-Pro, wherein X represents any amino acid residue, may be synthesized using solid-phase peptide synthesis, classic solution peptide synthesis, or oligomer-supported liquid-phase peptide synthesis. Classic solution peptide synthesis methods may be used with traditional purification steps and without the use of any polymer as a basis of attachment for the products as a method of separating the product from the unreacted reactants. Methods for carrying out liquid phase synthesis of libraries of peptides and oliqonucleotides coupled to a soluble oligomeric support have also been described. Bayer, Ernst and Mutter, Manfred, Nature 237:512-513 (1972) ; Bayer, Ernst, et al., J. Am. Chem. Soc. 96:7333-7336 (1974); Bonora, Gian Maria, et al., Nucleic Acids Res. 18:3155-3159 (1990). Both classic solution phase synthesis as well as oligomer-supported liquid phase synthesis, have the advantage over solid phase synthetic methods in that these synthesis methods do not require a structure present on a first reactant which is suitable for attaching the reactant to the solid phase. Also, classic solution phase synthesis methods do not require avoiding chemical conditions which may cleave the bond between the solid phase and the first reactant (or intermediate product). In addition, reactions in a homogeneous solution may give better yields and more complete reactions than those obtained in heterogeneous solid phase/liquid phase systems such as those present in solid phase synthesis.

In oligomer-supported liquid phase synthesis the growing product is attached to a large soluble polymeric group. The product from each step of the synthesis can then be separated from unreacted reactants based on the large difference in size between the relatively large polymer-attached product and the unreacted reactants. This permits reactions to take place in homogeneous solutions, and

homogeneous solutions, and eliminates tedious purification steps associated with classic solution phase synthesis.

For solid-phase peptide synthesis, the procedure entails the sequential assembly of the appropriate amino acids into a peptide of a desired sequence while the end of the growing peptide is linked to an insoluble support. Usually, the carboxyl terminus of the peptide is linked to a polymer from which it can be liberated upon treatment with a cleavage reagent. In a common method, an amino acid is bound to a resin particle, and the peptide generated in a stepwise manner by successive additions of protected amino acids to produce a chain of amino acids. Modifications of the technique described by Merrifield are commonly used. See, e.g., Merrifield, J. Am. Chem. Soc. 96: 2989-93 (1964), incorporated herein by reference in its entirety for all purposes. In an automated solid-phase method, peptides are synthesized by loading the carboxy-terminal amino acid onto an organic linker, which is covalently attached to an insoluble polystyrene resin cross-linked with divinyl benzene. Synthesis is accomplished in an automated peptide synthesizer, such as that available from Applied Biosystems. See, e.g., Model 430-A, Applied Biosystems, Foster City, Calif. Following synthesis, the product may be removed from the resin. A routine synthesis may produce 0.5 mmole of peptide-resin. Following cleavage and purification, a yield of approximately 60 to 70% is typically produced. Purification of the product peptides is accomplished by, for example, crystallizing the peptide from organic solvents or reverse high-pressure liquid chromatography (e.g., using a C.sup.18 column with 0.1% trifluoroacetic acid and acetonitrile as solvents). Purified peptide may be lyophilized and stored in a dry state until use. Analysis of the resulting peptides may be accomplished using the common methods of analytical high pressure liquid chromatography (HPLC) and electrospray mass spectrometry (ES-MS).

Acid addition salts of the present peptides can be prepared according to a conventional method. For example,

an acid addition salt can be obtained by reacting one of the present peptides containing a basic amino acid residue with a suitable acid in one equivalent amount thereto in water and then freeze-drying the product. Further, alkali or alkaline earth metal salts, ammonium salts or organic base salts (hereinafter these are referred to as base salts) can also be prepared according to a conventional method. For example, a base salt can be obtained by reacting one of the present peptides containing an acidic amino acid residue with a suitable base in one equivalent amount thereto in water and then freeze-drying the product.

The present peptides and their acid addition salts and base salts have a hypotensive activity, and thus are effective for treatment and/or prophylaxis of hypertension of mammals including human beings.

The present peptides and their acid addition salts or base salts are used alone or in the form of a food or pharmaceutical composition including at least one nutritional or pharmaceutical auxiliary. The present peptides and their acid addition salts and base salts can be administered parenterally, such as by intravenous injection or rectal administration, or orally, and formulated into a form suitable for each administration method.

Pharmaceutical forms for injections typically include sterilized aqueous solutions. Such formulations of the above form can further contain pharmaceutical auxiliaries other than water such as a buffering and a pH adjusting agent (for example, sodium hydrogenphosphate, citric acid), a tonicity agent (e.g., sodium chloride, glucose), or a preservative (methyl p-hydroxybenzoate, propyl-hydroxybenzoate or the like). The formulations can be sterilized by filtration through a bacteria-holding filter, by incorporation of a sterilant into the composition, or by irradiation or heating of the composition. The formulations can also be prepared as a sterilizing solid composition and dissolved at the time of use in sterilized water to form a solution for administration to the subject.

Orally administered agents are prepared in a form suitable for absorption in gastrointestinal organs. Food compositions contain at least one nutritional substance as a food auxiliary. Tablets, capsules, granules, fine granules and powders can contain conventional pharmaceutical auxiliaries, such as, a binder (e.g., syrup, gum arabic, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone or hydroxycellulose), an excipient (e.g., lactose, sucrose, corn starch, calcium stearate, sorbitol or glycine), a lubricant (e.g., magnesium stearate, talc, polyethylene glycol or silica), a disintegrant (potato starch or carboxymethylcellulose), or a wetting agent (e.g., sodium lauryl sulfate). Tablets can be coated using any conventional method. Oral liquid agents can be aqueous solutions or the like, or dry products to be dissolved at the time of use to create a solution. Such oral liquid agents may contain conventional additives such as a preservative (methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid or the like).

The anti-hypertensive peptides and their acid addition salts or base salts can also be ingested as an additional ingredient contained in foods and drinks, or as part of a functional food or health food. Further these peptides can be formulated together with nutrients such as various vitamins and minerals, into liquid foods such as nutrient drinks, soy milks and soups or solid foods of various types, or used in the form of a powder or by incorporation into various foods. The content of the effective anti-hypertensive ingredient in such a functional or health food can be similar to the dose contained in a typical pharmaceutical agent.

The amount of the present peptide or an acid addition salt or base salt thereof in the present anti-hypertensive agent can be varied, but it is preferred that the amount of peptide or acid addition salt or base salt in the agent be 1 to 100% (w/w), preferably 10 to 100% (w/w). When administered to a human being, the preferred amount for

administration of the effective ingredients of the present anti-hypertensive agent or ACE inhibitor is 1 to 200 mg/kg/day. Also, the preferred dosage is 1 to 200 mg/kg/day for the administration of the peptides of this invention for the method of inhibition of ACE and for the method of treatment or prophylaxis of hypertension.

All publications, patents, patent applications and other references cited in this application are herein incorporated by references in their entirety as if each individual publication, patent, patent application or other reference were specifically and individually indicated to be incorporated by reference.

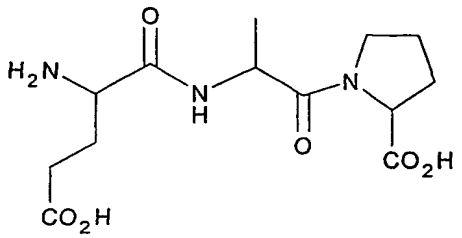
In one preferred embodiment, the tripeptides of the present invention are synthesized according to the procedure outlined below. The following examples are intended to illustrate but not limit the present invention.

EXAMPLES

Synthesis of Asp-Lys-Pro, Glu-Lys-Pro, Tyr-Lys-Pro, His-His-Pro, Glu-Ala-Pro, and Asp-Ala-Pro

Example 1

Glutamyl-Alanyl-Proline



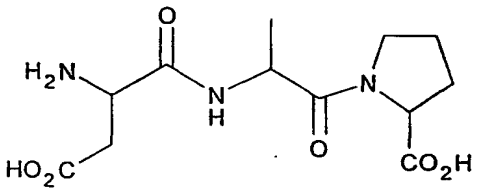
To a solution of Ala-Pro (0.5 g, 2.7 mmol) in dimethyl-formamide (DMF) (5 ml) was added DIEA (0.47 ml, 2.7 mmol). Then Boc-Glu(OtBu)-OSu (1.1 g, 2.7 mmol) and DMAP (50 mg) were added, respectively. After allowing the reaction to proceed overnight, the mixture was evaporated to dryness under reduced pressure at 35°C. The desired intermediate of N^{α}-Boc-γ-tert-Butyl-Glutamyl-Alanyl-Proline was isolated by

prep High performance liquid chromatography (HPLC) and lyophilized as white powder. The material was treated with 90% triflouro acetic acid (TFA) in water (10 ml) for 1.5 hours to give de-protected tripeptide which was further purified by prep HPLC and lyophilized as white powder (0.56 g).

ES-MS m/z 315 $(M+H)^+$.

Example 2

Aspartyl-Alanyl-Proline

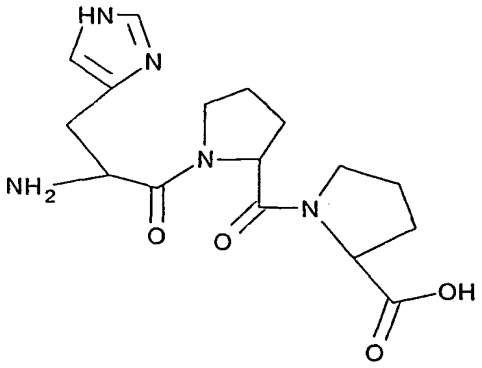


Aspartyl-Alanyl-Proline (0.62 g, 80% isolated yield) was prepared using substantially the same procedure as described in Example 1.

ES-MS m/z 301 $(M+H)^+$.

Example 3

Histidyl-Prolyl-Proline



Step 1:

Preparation of N^{α}-Boc-N^{im}-di-Boc-Histidyl-Prolyl-Proline

To a solution of HCl .H-Pro-Pro-OH (1.87 g, 2.5 mmol) in dimethyl formamide(DMF) (10mL) was added DIEA (0.97 g, 2.5 mmol). The glue like material thus formed was stirred at room temperature under N_2 for 0.5 h. Boc-His(Boc)-OSu (1.13 g, 2.5 mmol) in dimethyl-formamide (DMF) (15 mL, anydrous) solution was then added dropwise to the above solution. DMAP (100 mg) was added and the mixture was stirred at room temperature under N_2 for 5 days. After removal of the solvent, Boc-His(Boc)-Pro-Pro was purified by prep-HPLC, lyophilized and used directly in the next step.

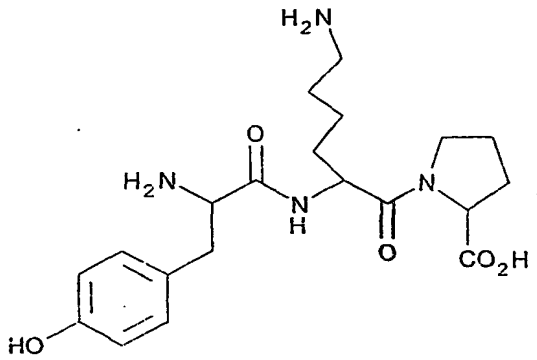
Step 2: Preparation of Histidyl-Prolyl-Proline

A solution of 4 N HCl in 1,4-dioxane (10 mL) was added to a solution of Boc-His(Boc)-Pro-Pro in the same solvent (10 mL, anhydrous). The mixture was stirred at room temperature under N_2 for 1 hour. The volatile was removed under reduced pressure. The resulting yellow oil was then purified by prep-HPLC to obtain 2TFA.His-Pro-Pro (470 mg, 32.6% overall yield).

ES-MS:m/z 349.9 $(M+H)^+$

Example 4

Tyrosyl-Lysyl-Proline



Step 1: Preparation of Nε-Boc-Lysyl-Proline-t-Butyl Ester

To a 0°C, stirred solution of Fmoc-Lys(Boc)-OH 94.68 g, 10 mmol) and BOBt (1.35 g, 10 mmol) in DMF (20mL) was added H-Pro-OtBu (1.71 g, 10 mmol) and EDC (1.91 g, 10 mmol). The mixture was stirred at room temperature for 8 hours and diisopropylamine (15% in DMF, 10 mL) was added. After the mixture was stirred for another 2 hours, the solvent was removed. The residue was dissolved in ethyl acetate, washed with H_2O, HCl (5%), K_2CO_3 (5%) and brine. The crude product was used for the next step.

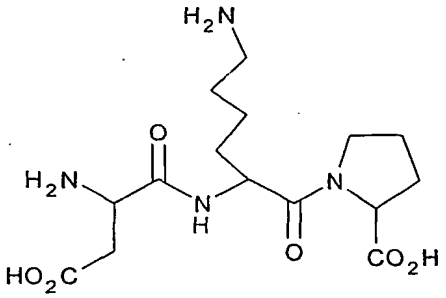
Step 2: Preparation of Tyrosyl-Lysyl-Proline

To a stirred solution of H-Lys(Boc)-Pro-OtBu (1.7 mmol) and Boc-Tyr-OH (0.57 g, 1.7 mmol) in DMF (10 mL) was added HOBt (0.26 g, 2 mmol), HBTU (0.76 g, 2 mmol) and DIEA (0.52 g, 4 mmol). The mixture was stirred at room temperature overnight. After the solvent was removed, Boc-Tyr-Lys(Boc)-Pro-OtBu was purified by prep-HPLC and lyophilized. The protecting groups were removed with a mixture of TFA/CH_2Cl_2 (V/V=95/5), purified by prep-HPLC and lyophilized to afford 2TFA.Tyr-Lys-Pro (0.42 g, 40% overall yield) as a white solid.

ES-MS: m/z 407.2 $(M+H)^+$.

Example 5

Aspartyl-Lysyl-Proline



Step 1: Preparation of Nε-Boc-Lysyl-Proline-t-Butyl Ester

To a 0°C, stirred solution of Fmoc-Lys(Boc)-OH 94.68 g, 10 mmol) and BOBt (1.35 g, 10 mmol) in DMF (20mL) was added H-Pro-OtBu (1.71 g, 10 mmol) and EDC (1.91 g, 10 mmol). The mixture was stirred at room temperature for 8 hours and diisopropylamine (15% in DMF, 10 mL) was added. After the mixture was stirred for another 2 hours, the solvent was removed. The residue was dissolved in ethyl acetate, washed with H_2O, HCl (5%), K_2CO_3 (5%) and brine. The crude product was used for the next step.

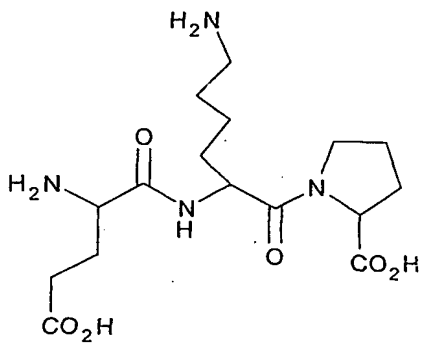
Step 2: Preparation of Aspartyl-Lysyl-Proline

To a stirred solution of H-Lys(Boc)-Pro-OtBu (1.7 mmol) and Boc-Asp-OH (1.7 mmol) in DMF (10 mL) was added HOBt (0.26 g, 2 mmol), HBTU (0.76 g, 2 mmol) and DIEA (0.52 g, 4 mmol). The mixture was stirred at room temperature overnight. After the solvent was removed, Boc-Asp-Lys(Boc)-Pro-OtBu was purified by prep-HPLC and lyophilized. The protecting groups were removed with a mixture of TFA/CH_2Cl_2 (V/V=95/5), purified by prep-HPLC and lyophilized to afford 2TFA.Asp-Lys-Pro (0.32 g, 34% overall yield) as a white solid.

ES-MS: m/z 359.1 $(M+H)^+$.

Example 6

Glutamyl-Lysyl-Proline



Step 1: Preparation of Nε-Boc-Lysyl-Proline-t-Butyl Ester

To a 0°C, stirred solution of Fmoc-Lys(Boc)-OH 94.68 g, 10 mmol) and BOBt (1.35 g, 10 mmol) in DMF (20mL) was added H-Pro-OtBu (1.71 g, 10 mmol) and EDC (1.91 g, 10 mmol). The mixture was stirred at room temperature for 8 hours and diisopropylamine (15% in DMF, 10 mL) was added. After the mixture was stirred for another 2 hours, the solvent was removed. The residue was dissolved in ethyl acetate, washed with H_2O, HCl (5%), K_2CO_3 (5%) and brine. The crude product was used for the next step.

Step 2: Preparation of Glutamyl-Lysyl-Proline

To a stirred solution of H-Lys(Boc)-Pro-OtBu (1.7 mmol) and Boc-Glu-OH (1.7 mmol) in DMF (10 mL) was added HOBt (0.26 g, 2 mmol), HBTU (0.76 g, 2 mmol) and DIEA (0.52 g, 4 mmol). The mixture was stirred at room temperature overnight. After the solvent was removed, Boc-Glu-Lys(Boc)-Pro-OtBu was purified by prep-HPLC and lyophilized. The protecting groups were removed with a mixture of TFA/CH_2Cl_2 (V/V=95/5), purified by prep-HPLC and lyophilized to afford 2TFA.Glu-Lys-Pro (0.33 g, 34% overall yield) as a white solid.

ES-MS: m/z 373.1 $(M+H)^+$.

Example 7

<u>In vivo Study of Anti-hypertensive Activity of the Peptides</u>

Spontaneously hypertensive rats (SHR) (Taconic, Germantown, New York, 6 animals per group, about 500g each) were used as test animals to examine the anti-hypertensive effects of intravenously administered synthetic peptides such as X-Pro-Pro, X-Ala-Pro, and X-Lys-Pro, wherein X represents any amino acid residue. Catheters were placed in the femoral veins of the animals for intravenous infusion. Ten μmoles of various synthetic peptides such as X-Pro-Pro, X-Ala-Pro, and X-Lys-Pro, wherein X represents any amino

acid residue, including Asp-Lys-Pro, Asp-Ala-Pro, Tyr-Lys-Pro, Glu-Ala-Pro, Glu-Lys-Pro, His-Pro-Pro, and Gly-Lys-Pro were intravenously administered to the SHR. The change in blood pressure immediately following IV administration was compared with that of a control group to which physiological saline was intravenously administered. Blood pressure was monitored continuously throughout the experiment through a catheter placed in the femoral artery using a polygraph. The results of these tests for the peptides of this invention are shown in the following table:

Table 1

Synthesized Peptides Which Significantly Reduce BP in SHR Rats

Compound	Mean SBP Lowering (mmHg)
EAP (Glu-Ala-Pro)	19.9
EKP (Glu-Lys-Pro)	36.5
DAP (Asp-Ala-Pro)	16.4
DKP (Asp-Lys-Pro)	20.8
YKP (Tyr-Lys-Pro)	17.7
HPP (His-Pro-Pro)	32.5

$p<0.05$; $\#0.1<p<0.05$ $R=0.115$

Example 8

Dose Response Studies

Dose response studies of the tripeptides Asp-Lys-Pro, Asp-Ala-Pro, Tyr-Lys-Pro, Glu-Ala-Pro, Glu-Lys-Pro, His-Pro-Pro, and Gly-Lys-Pro were performed on spontaneous hypertensive rats (SHR) with six (6) rats in each test group. Each testing peptide was administered in dosages of 0, 0.3, 1, 3, 10, and 30 umols/rat of each as well as 10umols/rat of Enalapril as a positive control in a group of six (6) rats, and the blood pressure was measured in these SHR at four (4) hours after oral administration of these peptides and Enalapril by tail cuff method using a noninvasive blood

pressure apparatus (Twelve channel Semi-Automatic NIBP Test System, IITC Inc.). The doses were delivered orally in about 1 ml volume. The result of this study was that all of the tripeptides administered significantly decreased blood pressure in these SHR. The maximal blood pressure lowering observed was about 25-30 mmHg. Graphic results of this study can be seen in Figures 1-5. The results of these tests for the peptides of this invention are shown in the following Tables.

Table 2

Effects of DKP on Systolic Blood Pressure of SHR

Dose (μmol)	Base-line Ave (mmHg)	SD	CV	4 Hr. Ave (mmHg)	SD	CV	4 Hr. Dif. (mmHg)
H_2O	190.04	13.804	0.0726	191.21	14.457	0.0756	1.1694
Enalapril (10 μmol)	191.78	11.49	0.0599	159.59	14.332	0.0898	-32.183
DKP (30 μmol)	193.79	18.693	0.0965	168.54	8.7314	0.0518	-25.253
DKP (10 μmol)	202.04	8.8522	0.0438	185.88	10.835	0.0583	-16.164
DKP (3 μmol)	195.63	20.456	0.1046	184.19	9.0844	0.0493	-11.433
DKP (1 μmol)	193.85	8.0929	0.0417	180.72	10.672	0.0591	-13.131
DKP (0.3 μmol)	190.71	22.502	0.118	189.5	12.049	0.0636	-1.2103

SD = Standard Deviation

CV = Coefficient of Variation

Table 3

Effects of YKP of Systolic Blood Pressure of SHR

Dose (μmol)	Base-line Ave (mmHg)	SD	CV	4 Hr. Ave (mmHg)	SD	CV	4 Hr. Dif. (mmHg)
H_2O	190.04	13.804	0.0726	191.21	14.457	0.0756	1.1694
Enalapril (10 μmol)	191.78	11.49	0.0599	159.59	14.332	0.0898	-32.183
YKP (30 μmol)	200.17	15.15	0.0775	175.73	11.727	0.0667	-24.442
YKP (10 μmol)	199.75	17.787	0.089	176.55	12.499	0.0708	-23.2
YKP (3 μmol)	198.42	18.369	0.0926	180.32	12.813	0.0711	-18.1
YKP (1 μmol)	198.99	18.738	0.0942	178.92	17.757	0.0992	-20.072
YKP (0.3 μmol)	191.99	13.34	0.0895	175.7	15.928	0.0907	-16.293

SD = Standard Deviation

CV = Coefficient of Variation

Table 4

Effects of HPP of Systolic Blood Pressure of SHR

Dose (μmol)	Base-line Ave (mmHg)	SD	CV	4 Hr. Ave (mmHg)	SD	CV	4 Hr. Dif. (mmHg)
H_2O	205.7	6.6023	0.0321	200.54	9.7379	0.0486	-5.1623
Enalapril (10 μmol)	212.77	15.086	0.0709	173.92	26.529	0.1525	-38.854
HPP (30 μmol)	206.32	17.962	0.0871	177.52	19.495	0.1098	-28.797
HPP (10 μmol)	205.83	13.685	0.0665	182.21	12.146	0.0667	-23.628
HPP (3 μmol)	200.13	9.8667	0.0493	179.01	13.958	0.078	-21.118
HPP (1 μmol)	206.26	14.424	0.0699	197.74	7.1202	0.036	-8.5213
HPP (0.3 μmol)	208,48	14.385	0.069	203.76	11.22	0.0551	-4.7236

SD = Standard Deviation

CV = Coefficient of Variation

Table 5

Effects of EKP of Systolic Blood Pressure of SHR

Dose (μmol)	Base-line Ave (mmHg)	SD	CV	4 Hr. Ave (mmHg)	SD	CV	4 Hr. Dif. (mmHg)
H_2O	205.7	6.6023	0.0321	200.54	9.7379	0.0486	-5.1623
Enalapril (10 μmol)	212.77	15.086	0.0709	173.92	26.529	0.1525	-38.854
EKP (30 μmol)	204.75	4.6566	0.0228	178.28	13.095	0.0735	-26.463
EKP (10 μmol)	201.61	16.032	0.0795	172.66	17.775	0.1029	-28.947
EKP (3 μmol)	215.5	16.177	0.0751	187.22	11.213	0.0599	-28.275
EKP (1 μmol)	202.66	13.705	0.0676	190.28	13.179	0.0693	-12.381
EKP (0.3 μmol)	206.87	23.994	0.116	196.39	16.046	0.0817	-10.486

SD = Standard Deviation

CV = Coefficient of Variation

Table 6

Effects of EAP of Systolic Blood Pressure of SHR

Dose (μmol)	Base-line Ave (mmHg)	SD	CV	4 Hr. Ave (mmHg)	SD	CV	4 Hr. Dif. (mmHg)
H_2O	194.59	15.663	0.0805	196.64	6.8438	0.0348	2.0444
Enalapril (10 μmol)	203.81	5.6527	0.0277	172.81	18.748	0.1085	-31.007
EAP (30 μmol)	202.45	19.145	0.0946	174.97	16.079	0.0919	-27.479
EAP (10 μmol)	203.17	13.031	0.0641	187.23	13.363	0.0714	-15.937
EAP (3 μmol)	201.49	13.247	0.0657	188.79	17.995	0.0953	-12.704
EAP (1 μmol)	205.81	13.244	0.0643	195.46	15.996	0.0818	-10.428
EAP (0.3 μmol)	215.23	15.269	0.0709	205.27	10.105	0.0492	-9.9556

SD = Standard Deviation

CV = Coefficient of Variation

Table 7

Effects of DAP of Systolic Blood Pressure of SHR

Dose (μmol)	Base-line Ave (mmHg)	SD	CV	4 Hr. Ave (mmHg)	SD	CV	4 Hr. Dif. (mmHg)
H_2O	200.14	8.6849	0.0434	197.33	11.981	0.0607	-2.8056
Enalapril (10 μmol)	190.13	31.354	0.1649	171.2	11.236	0.0656	-18.927
DAP (30 μmol)	206.85	18.098	0.0875	171.47	27.416	0.1599	-35.378
DAP (10 μmol)	212.25	14.896	0.0702	175.03	26.808	0.1532	-37.222
DAP (3 μmol)	209.21	14.489	0.0693	180.13	30.625	0.17	-29.082
DAP (1 μmol)	218.82	25.778	0.1178	196.76	27.533	0.1399	-22.064
DAP (0.3 μmol)	203.21	10.244	0.0504	183.6	19.801	0.1078	-19.605

SD = Standard Deviation

CV = Coefficient of Variation

In light of the detailed description of the invention and the examples presented above, it can be appreciated that the several aspects of the invention are achieved.

It is to be understood that the present invention has been described in detail by way of illustration and example in order to acquaint others skilled in the art with the invention, its principles, and its practical application. Particular formulations and processes of the present invention are not limited to the descriptions of the specific embodiments presented, but rather the descriptions

and examples should be viewed in terms of the claims that follow and their equivalents. While some of the examples and descriptions above include some conclusions about the way the invention may function, the inventors do not intend to be bound by those conclusions and functions, but put them forth only as possible explanations.

It is to be further understood that the specific embodiments of the present invention as set forth are not intended as being exhaustive or limiting of the invention, and that many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the foregoing examples and detailed description. Accordingly, this invention is intended to embrace all such alternatives, modifications, and variations that fall within the spirit and scope of the following claims.

What is claimed is:

1. A compound selected from the group of polypeptides consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof.

2. A peptide in substantially pure form selected from the group of peptides having the amino acid sequence Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro.

3. A pharmaceutical composition comprising an anti-hypertensively effective amount of at least one polypeptide selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof and a pharmaceutically acceptable carrier.

4. A composition of claim 3 wherein the composition is in an amount effective to reduce hypertension in a mammal.

5. A composition of claim 4 wherein the mammal is human.

6. A food composition suitable for treatment or prophylaxis of hypertension, the food composition comprising a nutritional substance and a compound selected from the group of polypeptides consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof, in an effective amount to produce an anti-hypertensive affect in a mammalian subject.

7. A food composition of claim 6, wherein the mammalian subject is human.

8. A food composition of claim 6, which is substantially a liquid.

9. A food composition of claim 6, which is substantially a gelatin.

10. A food composition of claim 6, which is substantially a solid.

11. A food composition of claim 6, which is a milk-based composition.

12. A food composition of claim 6, wherein the nutritional substance comprises a member selected from the group consisting of cooking oil, margarine, butter, mayonnaise, salad dressing and shortening.

13. An ingestible composition comprising the composition of claim 3 wherein the ingestible composition is selected from the group consisting of beverages, infused foods, sauces, condiments, salad dressings, fruit juices, syrups, desserts, icings and fillings, soft frozen products, confections, chocolates, candies, chewing gum and intermediate food.

14. A method for treatment and prophylaxis of hypertension in a subject, said method comprising administering to the subject an anti-hypertensively effective amount of the composition of claim 3.

15. A method for treatment and prophylaxis of hypertension of a human being or other mammal which comprises administering to the human being or other mammal an anti-hypertensively effective amount of at least one polypeptide selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, and His-Pro-Pro and a pharmacologically acceptable acid or base

addition salt thereof and a pharmaceutically acceptable carrier.

16. A method for treatment and prophylaxis of myocardial infarction caused by or associated with hypertension of a human being or other mammal which comprises administering to the human being or other mammal an anti-hypertensively effective amount of at least one member selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof and a pharmaceutically acceptable carrier.

17. A method for treatment and prophylaxis of left ventricular systolic dysfunction caused by or associated with hypertension of a human being or other mammal which comprises administering to the human being or other mammal an anti-hypertensively effective amount of at least one member selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof and a pharmaceutically acceptable carrier.

18. A method for treatment and prophylaxis of renal impairment or failure caused by or associated with hypertension of a human being or other mammal which comprises administering to the human being or other mammal an anti-hypertensively effective amount of at least one member selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof and a pharmaceutically acceptable carrier.

19. A method for treatment and prophylaxis of diabetes mellitus caused by or associated with hypertension of a human being or other mammal which comprises administering to the human being or other mammal an anti-hypertensively

effective amount of at least one member selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof and a pharmaceutically acceptable carrier.

20. A method for treatment and prophylaxis of congestive heart failure caused by or associated with hypertension of a human being or other mammal which comprises administering to the human being or other mammal an anti-hypertensively effective amount of at least one member selected from the group consisting of Asp-Lys-Pro, Tyr-Lys-Pro, Glu-Lys-Pro, Asp-Ala-Pro, Glu-Ala-Pro, His-Pro-Pro and a pharmacologically acceptable acid or base addition salt thereof and a pharmaceutically acceptable carrier.

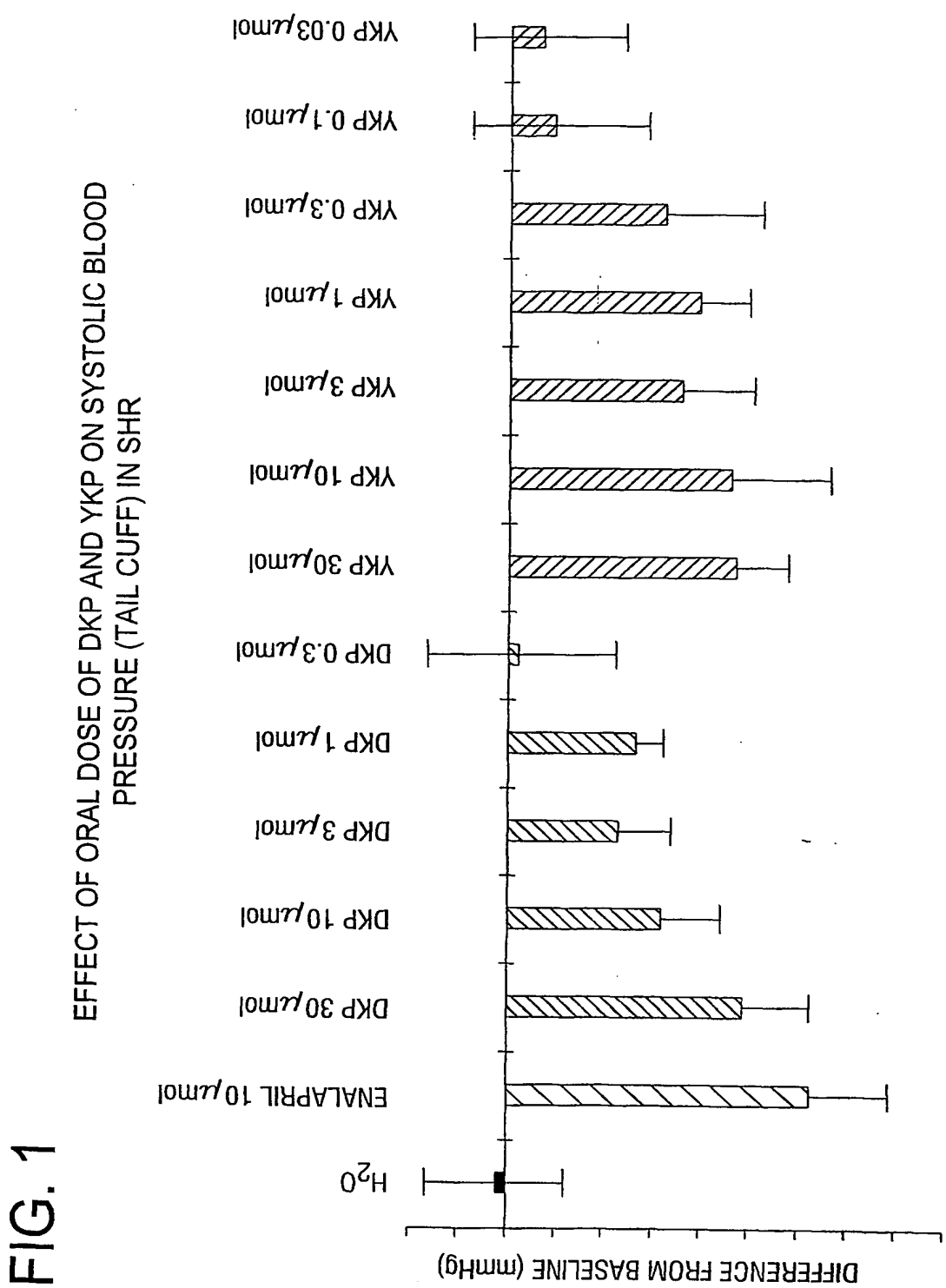

EFFECT OF ORAL DOSE OF DKP AND YKP ON SYSTOLIC BLOOD PRESSURE (TAIL CUFF) IN SHR
DIFFERENCE FROM BASELINE (mmHg)
H2O
ENALAPRIL 10μmol
DKP 30μmol
DKP 10μmol
DKP 3μmol
DKP 1μmol
DKP 0.3μmol
YKP 30μmol
YKP 10μmol
YKP 3μmol
YKP 1μmol
YKP 0.3μmol
YKP 0.1μmol
YKP 0.03μmol

FIG. 1

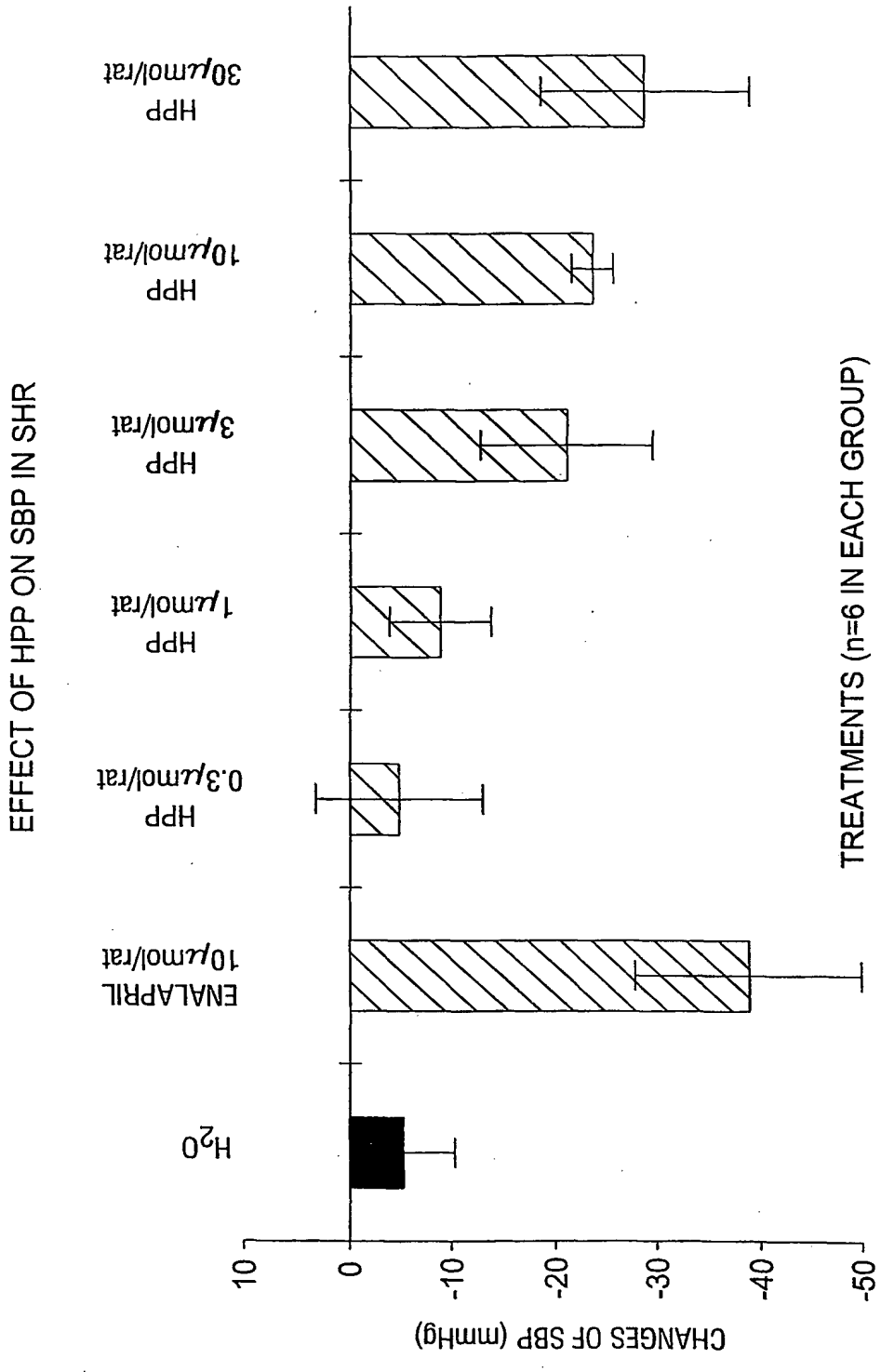

EFFECT OF HPP ON SBP IN SHR
CHANGES OF SBP (mmHg)
10
0
-10
-20
-30
-40
-50
TREATMENTS (n=6 IN EACH GROUP)
H2O
ENALAPRIL 10μmol/rat
HPP 0.3μmol/rat
HPP 1μmol/rat
HPP 3μmol/rat
HPP 10μmol/rat
HPP 30μmol/rat

FIG. 2

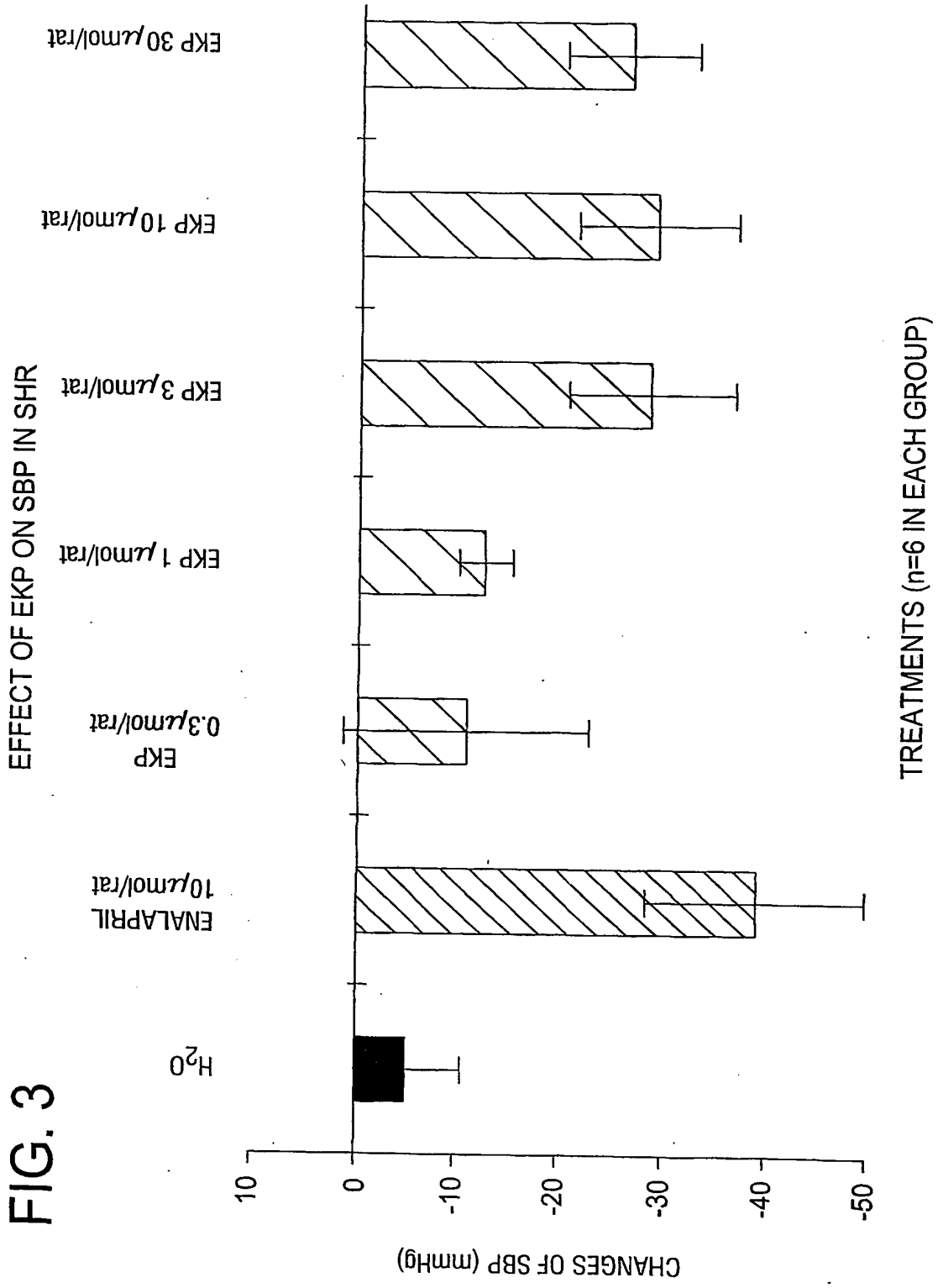
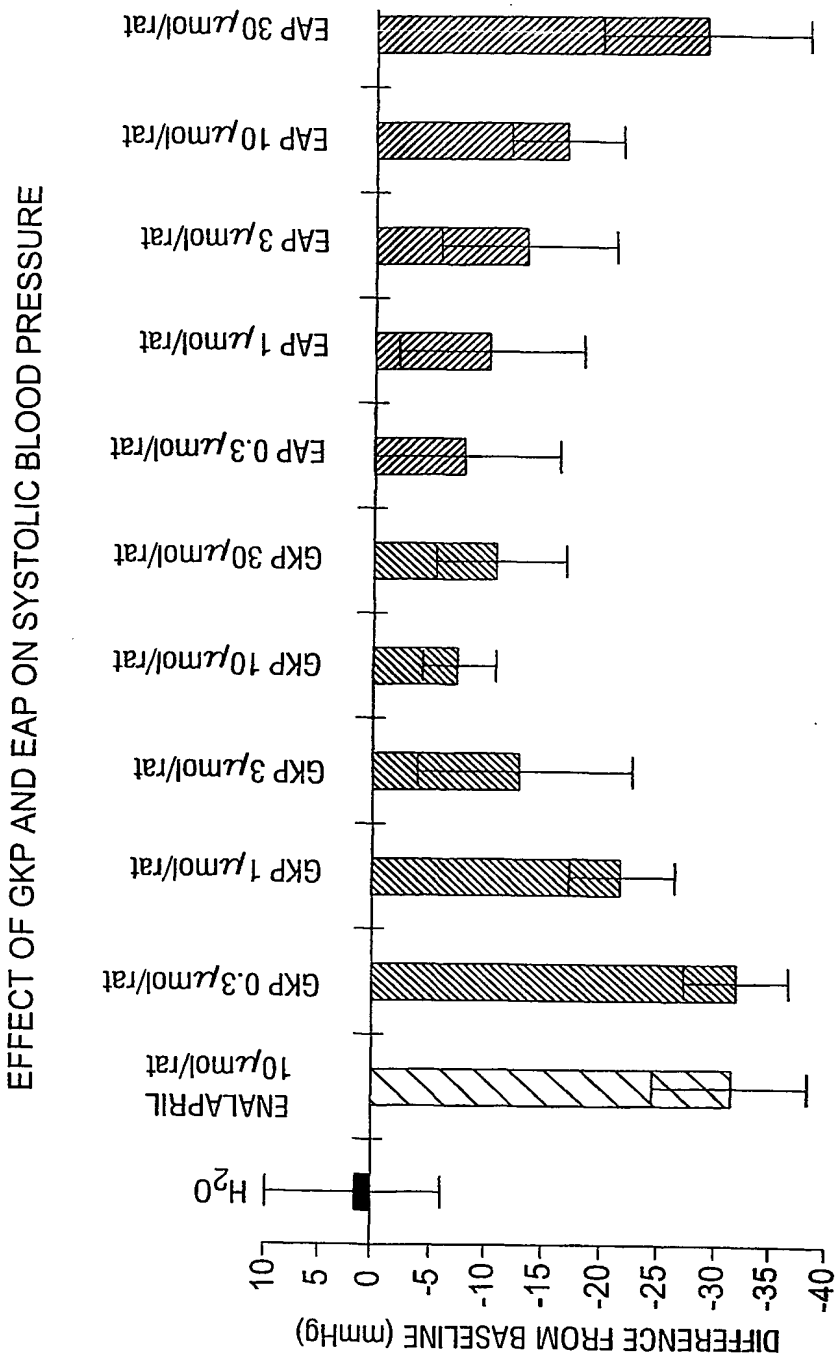

EFFECT OF EKP ON SBP IN SHR
CHANGES OF SBP (mmHg)
10
0
-10
-20
-30
-40
-50
TREATMENTS (n=6 IN EACH GROUP)
H2O
ENALAPRIL 10μmol/rat
EKP 0.3μmol/rat
EKP 1 μmol/rat
EKP 3μmol/rat
EKP 10μmol/rat
EKP 30μmol/rat

FIG. 3

FIG. 4


EFFECT OF GKP AND EAP ON SYSTOLIC BLOOD PRESSURE
DIFFERENCE FROM BASELINE (mmHg)
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
H2O
ENALAPRIL 10μmol/rat
GKP 0.3μmol/rat
GKP 1μmol/rat
GKP 3μmol/rat
GKP 10μmol/rat
GKP 30μmol/rat
EAP 0.3μmol/rat
EAP 1μmol/rat
EAP 3μmol/rat
EAP 10μmol/rat
EAP 30μmol/rat

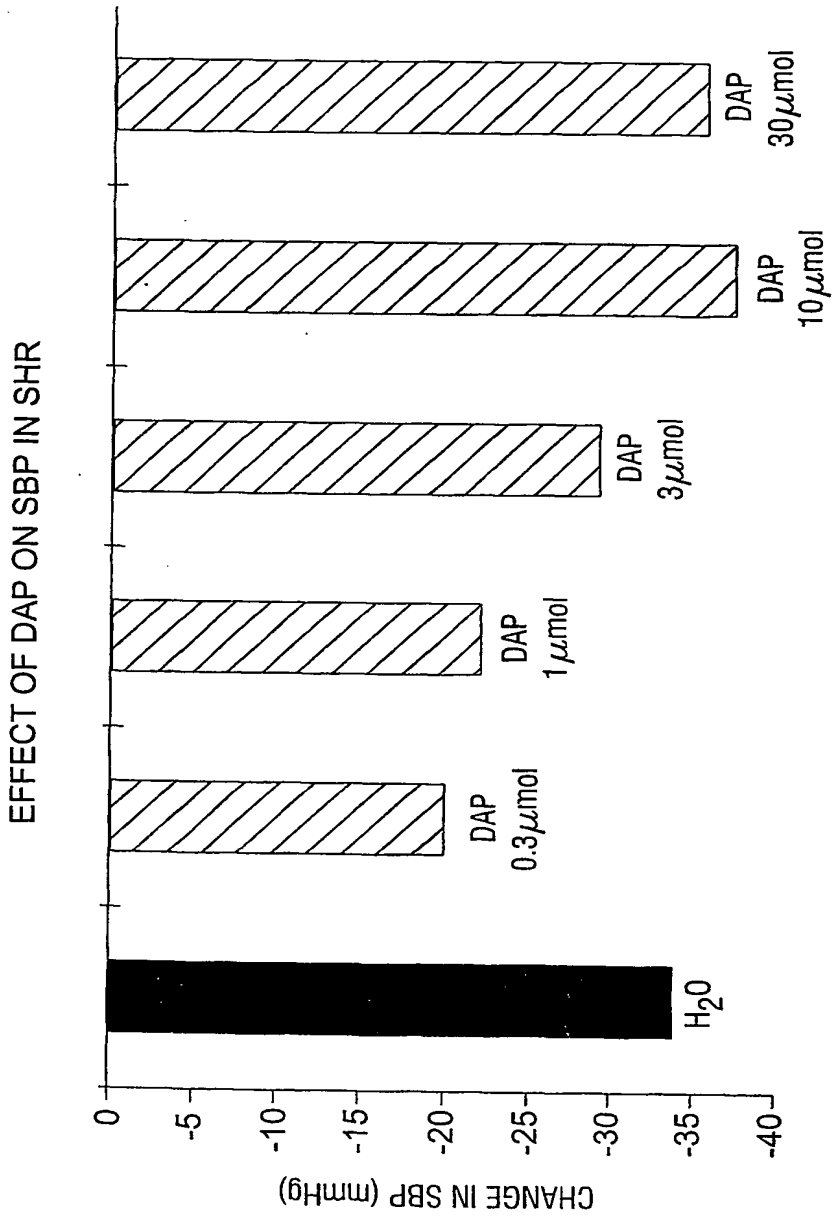

EFFECT OF DAP ON SBP IN SHR
CHANGE IN SBP (mmHg)
0
-5
-10
-15
-20
-25
-30
-35
-40
H_2O
DAP 0.3μmol
DAP 1μmol
DAP 3μmol
DAP 10μmol
DAP 30μmol

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/07530

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 38/06; C07 K 5/00, 5/10; A23C 9/12

US CL : 514/2, 18; 530/331, 344, 354; 426/ 34, 580, 590, 656

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 18; 530/331, 344, 354; 426/ 34, 580, 590, 656

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MONTECUCCHI et al., Primary Structure Determination of a Tryptophan-Containing Tridecapeptide from Phyllomedusa rohdei. Int. J. Peptide Protein Res. 1986, Vol 27, pages 175-182. See abstract, Scheme 1.	2
X	ZAIDI et al., Amino Acid Sequence of a Peptide from the Hypothalamus of Rana cyanophlyctis. Int. J. Peptide Protein Res. 1981, Vol 18, pages 516-518. See pages 516-518.	2
X	FIAT et al., Specificity of Renin: Renin-Sensitive Sequence of Sheep kappa-casein. Chimia 1970, Vol 24, page 220. See page 220.	2
Y	NAKAMURA et al., Antihypertensive Effect of Sour Milk and Peptides Isolated from It That Are Inhibitors to Angiotensin I-Converting Enzyme. J. Diary Sci. 1995, Vol 78, pages 1253-1257. See abstract, page 1254-1255, Fig. 1, 2.	3, 4, 6, 8, 11-15
Y	US 5,854,029 (CALPIS CO., LTD.) 29 December 1998. See columns 2-4, Examples 1-3.	3, 4, 6, 8, 10-15
A	US 5,691,310 (VESELY) 25 November 1997. See columns 3-6, Figs 2-5, Examples.	14, 15, 18, 20
A,P	CASARINI et al., Angiotensin Converting Enzymes from Human Urine of Mild hypertensive Untreated Patients Resemble the N-Terminal Fragment of Human Angiotensin I-Converting Enzyme. Int. J. Biochem. Cell Biology January 2001, Vol 33 [illegible] See the whole document.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 July 2001(15.07.2001)

Date of mailing of the international search report

07 AUG 2001

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

Mr. KAM

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/07530

Continuation of B. FIELDS SEARCHED Item3:
STN search on CAPLUS, MEDLINE, EMBASE, BIOSIS and SCISEARCH; EAST search on USPAT, DERWENT, EPO, JPO. Search term used: antihypertensive, hypertensive, hypertension, polypeptide, peptide, treating, treatment, prophylaxis, composition, food, ingestible, myocardial infarction, human, mammal, ventricular systolic dysfunction, renal failure, renal impairment, diabetes mellitus, congestive heart failure. Amino acid sequence search for Asp-Lys-Pro, Tyr-Lys-pro, Glu-Lys-Pro, Glu-Ala-Pro, Asp-Ala-Pro, His-Pro-Pro.